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MEETING REVIEW

Biofilms 2009: New Perspectives at the Heart of Surface-Associated Microbial Communities▽

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Biofilms have a major impact on human health, the environment, and industry. Thus, a better understanding of the mechanisms controlling biofilm formation and activity has the promise to transform the way physicians treat biofilm infections and to help us understand the activities of bacteria in the natural environment. For these reasons, biofilm research is currently an area of intense interest. The 5th ASM Conference on Biofilms brought together scientists from a wide range of scientific disciplines. Biofilms 2009 was held from 15 to 19 November 2009 in Cancun, Mexico. The conference was attended by 441 participants from a number of countries. Representing state and federal agencies, academic institutions, and professional organizations, the participants exchanged information and ideas on biofilm microbiology. Due to the international, multidisciplinary, and multicultural character of the meeting, the attendees had the opportunity to look beyond their own methodologies and praxis to novel technologies and experimental approaches. Most would agree that the meeting stimulated innovative thinking and new collaborations.

As in past meetings, keynote speakers were invited to set the stage for three key emerging biofilm-related themes. In addition, the conference program included talks by invited, established researchers, as well as those selected by the scientific organization committee from the poster abstracts. The presenters shared their perspectives from fields as varied as biology, physics, chemistry, engineering, bioinformatics, biotechnology, and medicine. The meeting included three extensive poster sessions where investigators had the opportunity to discuss their research. A new feature of this year's biofilm conference was the presentation of four poster awards. Three of them were awarded in memory and recognition of outstanding scientists whose lifelong dedication to biofilm research made them internationally recognized leaders in the field. The Bill Characklis Poster Award for Excellence in Engineering in Biofilm Research, the Terry Beveridge Poster Award for Excellence in Biofilm Microscopy, the Peter Gilbert Poster Award for Excellence in Innovation and Biofilm Control, and the Award for Outstanding Research Poster in the open category were presented to four distinguished young researchers at the conference banquet.

The evening specialty sessions on evolving interdisciplinary biofilm topics were a key feature of the conference. These sessions provided an interactive forum for attendees to discuss areas of interest. There were also hands-on workshops to experience and discuss innovations in methodology related to imaging and quantifying ex vivo biofilms and flow cell operations, the utility of animal models for studying biofilms, and to aim at standardizing biofilm methods for routine use. Participants indicated that there is a strong need to encourage collaborations between methodologists and basic and clinical scientists, as well as to standardize experimental approaches, which would allow for more robust and comparable results.

There were 12 sessions in the oral program. Special emphasis was placed on emerging topics in biofilm research. It is recognized that work on model organisms grown under standardized laboratory conditions has led to a better understanding of the molecular mechanisms associated with the biofilm mode of growth. However, the need to study biofilm communities *in situ* is increasingly appreciated. In habitats including the human host, bacteria tend to associate with other genera and form multispecies communities exhibiting increased levels of complex interactions. To address emerging areas of importance, the committee sought to draw attention to relevant multispecies biofilms and their interactions with the human host, to emerging technologies for the study of structured systems, to the latest research insights into regulatory and structural aspects of biofilm development, and to health strategies that can be applied to control and combat biofilm diseases.

In this review, we summarize the three keynote talks, as well as the individual sessions. We hope this compendium will provide the attendees of the Biofilm 2009 conference with a synopsis of the scientific highlights presented and update those who were unable to attend but are interested in surface-associated microbial life.

BIOFILMS AND THE HUMAN MICROBIOME

The opening keynote address was delivered by David Relman. He discussed concepts related to macroecology and how they could be applied to microbiology. One such concept is island biogeography, and he discussed how individual teeth

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and subgingival pockets might constitute their own "islands," with their own distinct microbial flora. He also discussed how oral communities might display a quasistable distribution of species and that "catastrophic" events in the oral cavity could cause a stable shift in the species composition, with potential ramifications for disease. His talk set the tone for the first session focused on the human microbiome.

The first scientific session was entitled Biofilms and the Human Microbiome and was chaired by Bill Costerton. Biofilms living in and on the human body have been investigated from clinical and bacteriological perspectives for decades and even, in the case of oral biofilms, since the late 17th century, beginning with van Leeuwenhoek's description of the animalcules scraped from his teeth. Modern investigations have approached these biofilms from the standpoint of colonization and infection and have focused on the question of what drives the transition between the two states. Bruce Paster spoke about the need to examine entire microbial communities rather than individual pathogens (36). In the complex disease of periodontitis, several keystone organisms seem to be critical for the stabilization of communities, and likewise, several pathogens are typically involved in their disturbance, thus triggering disease. Rapid, array-based technology now exists for profiling of oral communities and has revealed that community composition at periodontally diseased sites can differ on a tooth-by-tooth basis within a single individual.

Larry Forney presented results from a cross-sectional study on the vaginal community composition of healthy North American women from four ethnic groups. Seven community profiles with a distinct structure and composition could be distinguished; however, their relative frequencies significantly varied between racial groups (51, 53). Since the species composition of vaginal bacterial communities may affect the risk for developing bacterial vaginosis, inherent differences may have important consequences in risk assessment and disease diagnosis. Our bacterial symbionts interact with us on several levels, one of which is thought to be the innate immune system (19). Lora Hooper spoke on the relationship between bacterial colonization of the gut and the host's production of a C-type lectin that binds peptidoglycan (9). The lectin RegIIIγ was shown to be expressed by specialized secretory Paneth cells via the MyD88-dependent trigger in response to the presence of bacteria. RegIIIγ and other MyD88 factors are now being investigated as modulators of bacterium-host homeostasis within the gut along spatial boundaries, as well as during development. The session provided a broad-based overview of bacterial community structure and host-bacterium interactions in biofilms intrinsic to human health.

BIOFILM DEVELOPMENT

The session on biofilm development was chaired by Joshua Shrout and Alison Kraigsley. Jean-Marc Ghigo presented research exploring adhesion in *Escherichia coli* mediated by type I pili (or fimbriae) that are present in both laboratory strains (e.g., K-12) and pathogenic strains (45). Ghigo and colleagues have identified seven other type 1 fimbriae which are expressed in a Fim null background. The authors used a glycochip assay to determine that all of the pili have different ligand (sugar) specificities and that two do not bind sugar at all. They showed that all seven pili can be functionally expressed and have structural homology but have different functions. To survive when passing from the environment, through the host, and then back into the environment, *E. coli* might have developed different mechanisms for adherence in each of these specific environments. George O'Toole elaborated upon how bis-(3′,5′)-cyclic dimeric GMP (c-di-GMP) influences biofilm formation via sensing environmental phosphate levels in *Pseudomonas fluorescens*. Low phosphate was shown to limit biofilm formation through LapA, a biofilm adhesin. Further investigation of the influence of phosphate upon LapA identified a signaling cascade that involves three proteins, LapA, LapD, and LapG. Whereas LapA is a cell-associated protein that promotes adhesion, LapD is a c-di-GMP-sensing protein that performs "inside-out" signaling (34). LapG cleaves the N-terminal residues of LapA to form the active, cell-associated form of LapA when LapD is dissociated from LapG, which occurs under conditions of low phosphate or high c-di-GMP levels. This interesting regulatory cascade shows how environmental inputs can regulate biofilms.

Matthew Parsek talked about rugose small-colony variants (RSCV) of *Pseudomonas aeruginosa* which are found in both the cystic fibrosis lung and laboratory biofilms (41). RSCV have increased biofilm formation capacity and increased levels of the intracellular signaling molecule c-di-GMP. In RSCV, a c-di-GMP-induced extracellular adhesin, CdrA, is important for localization of the Psl polysaccharide in biofilms. It was found that biofilms formed by *cdrA* mutants lacked a protective Psl shell. CdrA was implicated in maintaining the integrity of biofilm communities through extracellular binding of Psl (8). Angela Nobbs presented work investigating *Candida albicans* adhesion in an oral environment through the heterologous expression of its adhesins. *C. albicans* adhesion proteins were expressed on the surface of *Saccharomyces cerevisiae* to determine if the *S. cerevisiae* cells would bind *Streptococcus gordonii*, denture materials, or saliva-coated host tissue. It was found that Als3 and Hwp1 were involved in binding to saliva-coated tissue. Additionally, Eap1 and Hwp1 were shown to be involved in adherence to synthetic surfaces and finally Als1 and Eap1 were required for interaction with *S. gordonii*.

Detailed analysis of gene regulation during biofilm development will help us to further understand the biofilm development process. To that aim, Kim Hermans presented the new technique of investigated differential fluorescence induction (DFI) as a way to examine gene regulation during biofilm formation by *Salmonella enterica* serovar Typhimurium (33). A fluorescent library with small DNA fragments cloned upstream of a promoterless green fluorescent protein gene was constructed, and fluorescence-activated cell sorting identified 19 individual cells that had a particular promoter activated under biofilm growth conditions (9 of which mapped to known promoters). DFI may be extremely useful in conjunction with bioinformatic techniques to monitor regulation within biofilms on a single-cell level.

Karin Sauer identified three two-component systems (TCS) in *P. aeruginosa* called BifSR, BfmSR and MifSR (37). Deletion of these three TCS factors arrested biofilm development at different stages but had no effect upon planktonic cells or initial attachment. BifSR acted early upon biofilm development, while BfmSR and MifSR acted at later stages. When TCS

expression was discontinued in mature biofilms, the biofilm phenotype reverted to that of the earlier time-specific stages. Dan Kearns presented his findings on the bifunctional EpsE protein of *Bacillus subtilis* (6). EpsE acts as both a clutch to inhibit flagellar rotation and an important component of exopolysaccharide (EPS) synthesis. As a clutch, EpsE interacts with FliG to disengage the *B. subtilis* flagellar motor. In addition, EpsE has sequence similarity to a glucosyltransferase enzyme. The *epsE* gene resides within an operon, under the control of SinR, that produces EPS, leading to attached biofilm cells. Dan Wozniak discussed the role of Psl polysaccharide during *P. aeruginosa* biofilm development. Psl is widely conserved across *Pseudomonas* species and was identified as a glucose-rhamnose-mannose pentasaccharide that promotes attachment of cells to both biotic and abiotic surfaces. Further inspection of Psl localization (using Psl-binding lectins) demonstrated a helical arrangement of Psl outside the *P. aeruginosa* cell, which may be important for cell-cell physical interactions and ordering of groups (52).

THE BIOFILM MATRIX

This session was cochaired by Rajendra Deora and Hans Curt Flemming. The EPS matrix remains on the radar of biofilm research, as it represents the immediate environment of biofilm cells, directly influencing their environment and the overall properties of biofilms. This can be seen with dental caries, which is an oral infectious disease that affects the majority of the world's population. Production of an EPS matrix by *Streptococcus mutans* from dietary carbohydrates is one of the principal mechanisms of the development of dental biofilms. Hyun Koo presented data illustrating complex and dynamic changes in the sequential assembly and structure of the EPS matrix during biofilm development (50). It appears that specific localization and spatial distribution of EPS are critical for dental biofilm development, leading to the proposal that EPS may represent a therapeutic target for the prevention of dental caries. For *Staphylococcus aureus*, Ken Bayles presented a model in which the metabolic heterogeneity present in the biofilm plays an important role in the differential control of *cid* and *lrg* expression within biofilm subpopulations (30). The *cid* operon encodes a holin which controls bacterial death and lysis, while the *lrg* operon encodes the antiholin. Thus, these operons determine the death and lysis of biofilm cells. Biofilm cell death can lead to the presence of extracellular DNA (eDNA) in the biofilm matrix. Alexander Horswill further elaborated on the role of eDNA in *S. aureus* biofilm development (24). He argued that eDNA seems to be a major EPS component, maintaining the integrity of the matrix, and is regulated by nuclease activity.

Shawn Lewenza investigated genes responsible for aggregation in *P. aeruginosa*. Again, eDNA was identified as a functional component of the matrix, playing an adhesive role in maintaining biofilm structure. In addition, it can act as a divalent metal cation chelator (32). He suggested a signaling role for eDNA in activating multiple metal-responsive two-component regulatory systems through chelation of these metals. The role of eDNA was hypothesized to be very important in promoting lung infections that cannot be cleared by immune response or antibiotic therapy. June Javen-Wolfe used *Caulobacter crescentus* as a model biofilm organism because it is easy to culture and synchronize and has a dimorphic lifestyle with an obligate adhesion phase (43). She presented data on holdfast, a polarly secreted adhesin of this organism. By studying mutants with deletions of genes involved in holdfast export and fluorescent protein fusions, it was demonstrated that the synthesis and export proteins interact in a multienzyme complex for optimal biosynthesis and attachment. Altogether, regulation of matrix formation and the role of eDNA were main focal elements of EPS research talked about at the meeting.

The second keynote address was given by Derek Lovley. Lovley described his efforts to understand and engineer the microbiology behind microbial fuel cells. One of the most effective current-producing bacteria is *Geobacter sulfurreducens* (15). Lovley demonstrated that the current output of a microbial fuel cell could be significantly increased by the accumulation of biomass on the anode surface and with increasing height of the anode biofilm. This finding implies that the cells are metabolically active throughout the biofilm and that cells even at a substantial distance from the anode surface contribute to current production. Lovley also introduced mechanisms for potential long-range electron transfer, which has to span at least 50 to 100 μm. Long-range electron transfer is probably due to a highly conductive biofilm matrix, largely mediated by electrically conductive pili. A detailed characterization of the phenotypic changes in the outer surface of the cell may provide valuable insights into the mechanisms of microbe-electrode interactions as the next step on the way to create *Geobacter*-based fuel cells that can generate ecologically responsive electricity.

INTRACELLULAR SIGNALING IN BIOFILM COMMUNITIES

This session was moderated by Alex Rickard and Robert J. C. (Bob) McLean. Intriguing multidisciplinary work presented by Jeremy Webb showed that both bacterial biofilms and cancer cell lines generate highly recalcitrant three-dimensional foci known as microcolonies in biofilms and as tumor spheroids in cancer cell models. Webb and coworkers showed that mutator phenotypes can enhance the development of three-dimensional structures in both bacterial biofilms and cancer cell foci (10). He proposed that the evolutionary and ecological constraints of growth in these taxonomically very different systems are fundamentally similar and thus can be addressed within the same experimental and conceptual framework.

One of the big issues related to biofilm growth is the involvement of quorum sensing and the onset of antibiotic resistance. Marvin Whiteley reported on some exciting work done in collaboration with Jason Shear and Jodie Connell in the chemistry department at the University of Texas. Using an elegant microscopic chamber, Dr. Whiteley was able to show that a few thousand bacteria were able to constitute a quorum (express quorum-regulated genes) and become antibiotic resistant. This small population size is analogous to the number of bacteria found in microbial clusters (microcolonies) that are a common feature of biofilms. Vanessa Sperando described how epinephrine and norepinephrine, through the QseC sensor, regulate virulence in enterohemorrhagic *E. coli in vitro* and during murine infection (40). The signaling cascade activates the expression of key virulence determinants such as the flagellar regulon

and type III secretion. This work emphasizes the ability of pathogenic bacteria to respond to specific host-derived signals.

Work was presented discussing the ability of signals to induce biofilm cells to enter the planktonic growth state. David Davies described a monounsaturated fatty acid, *cis*-2-decenoic acid (*cis*-DA), produced by cultures of *P. aeruginosa* (28). When added exogenously to continuous cultures, *cis*-2-decenoic acid was shown to induce cell-mediated disaggregation of biofilm microcolonies, releasing live planktonic bacteria into the surrounding bulk liquid. This molecule was also shown to induce dispersion of biofilms formed by a number of bacterial species. Furthermore, *cis*-DA was capable of inducing dispersion in biofilms of *C. albicans*, indicating that this molecular messenger has cross-kingdom functional activity. Finally, Laura Case, a Ph.D. student with Gary Dunny, showed how biofilm growth produced bistability in the population for pheromone-inducible conjugation in the important Gram-positive pathogen *Enterococcus faecalis*. Biofilm growth produced a subpopulation that was able to respond to very low concentrations of pheromone (the peptide cCF10) (18).

BIORESOURCES

Recently, biofilms have been applied in much larger scale engineering projects such as wastewater treatment and bioremediation and as a potential source of global energy. Biofilms can positively and negatively impact natural bioresources such as river systems and potable water distribution systems or become a bioresource in themselves through the production of energy in various forms, such as the direct production of electricity, the production of hydrocarbons, or as a source of biomass for biofuel. This session, chaired by Hilary Lappin-Scott and Paul Stoodley, explored various systems in which biofilms could either influence natural systems or be considered a bioresource in their own right. The first speaker was Harald Horn. The research had application in natural and manufactured flow systems. A long-term flow reactor experiment, run over 48 days, looked at the driving forces determining biofilm structure, ecology, and function while external conditions remained constant (temperature, nutrients, flow rate). For the first 48 days, the biofilm was relatively stable with periods of sloughing followed by regrowth in which the same type of biofilm was reestablished. However, at day 48 there was a "catastrophic switch" in which the biofilm became dominated by filamentous fungi. There appeared to be an overall decrease in biomass as the more compact biofilm was replaced by filaments and the increased mass transfer by the motion of the streamers compensated, so that the overall activity of the reactor, in terms of substrate conversion rate, remained the same.

Anne Camper focused on the relationship between microbial ecology and overall biofilm physiology in a drinking water distribution pipeline. The study looked at the influence of chlorite, which is used in the industry to control nitrification, on microbial ecology and overall biological activity. Chlorite eliminated nitrite-oxidizing bacteria, but ammonia-oxidizing bacteria persisted; also, the community became dominated by ammonia-oxidizing archaea and fungi; this, combined with the discovery of the *amoA* gene from a cultured *Pseudomonas* species, suggested that there is a much wider community of nitrifying organisms in the environment than previously considered. Ilia Baskakov talked about the potential use of cyanobacterial biofilms to produce electrical current. Denaturing gradient gel electrophoresis demonstrated that a diverse range of genera and cyanobacterial species were able to produce a voltage, and it was concluded that the growth system could be used as a screen for potential candidate strains in the optimization process. Scaling up from measurements of yields from the laboratory reactors suggested that it might be possible to generate electricity for use on a global scale. The final talk of the session was given by Laura Selan. Coagulase-negative staphylococci are utilized in the ripening process in various Italian cheeses for flavor and aroma. The study investigated the diversity of these strains in terms of adhesion, optimal growth temperature, and biofilm formation.

BIOFILMS IN MEDICAL AND DENTAL INFECTIONS I

In the first of the two sessions designed to describe the role of bacterial biofilms in chronic infectious diseases, the session chairs were Thomas Bjarnsholt and Luanne Hall-Stoodley. The presentations focused on clinically relevant biofilms, emphasizing the challenges that biofilms present due to their recalcitrance to antimicrobial treatment and host defenses, and presented novel treatment strategies for biofilm infections. The first seminar, given by Randy Wolcott, presented research on chronic wound infections, which are associated with over 50,000 deaths a year. Unlike acute wounds, chronic wounds display typical characteristics of biofilm infections: aggregated polymicrobial bacteria attached to the epidermis accompanied by intense neutrophilic inflammation (21). Using a clinical definable patient subgroup (patients with critical limb ischemia), 190 patients were subjected to either conventional wound care or a therapeutic regimen designed to reduce wound biofilm by a combination of debridement and antibiofilm treatment. Results indicated that wound management that targeted biofilms resulted in a statistically significant improvement in wound healing compared with standard wound care.

Lauren Bakaletz reported on recurrent chronic otitis media (OM). Nontypeable *Haemophilus influenzae* (NTHi), the predominant pathogen of chronic OM, forms a biofilm community within the middle ear cavity (2). She discussed the functional aspects of NTHi type IV pili in the experimental model of OM, the chinchilla. Immunization with recombinant soluble PilA induced antibodies that blocked twitching motility and inhibited both adherence and biofilm development. *In vivo* immunization correlated with significant protection against the development and severity of OM and rapidly resolved existing disease, including the presence of NTHi biofilm within the chinchilla middle ear space. Scott Hultgren outlined ongoing research on biofilm infections of the urinary tract (47). Using uropathogenic *E. coli* (UPEC) as a model system, the type 1 pilus adhesin FimH was shown to mediate UPEC specific binding to and entry into specialized cells lining the bladder. Specifically, FimH/mannose receptor binding was critical for disease progression, activating a complex genetic cascade in the bacterial pathogenic cycle that has been well characterized: formation of intracellular biofilms called intracellular bacterial communities. While host phagocytes such as polymorphonuclear neutrophils (PMNs) were able to kill unaggregated UPEC, they were unable to kill UPEC biofilms and therefore

both the intracellular niche and biofilm formation protect UPEC from host defenses. Experiments showed that *E. coli* cells were embedded in amyloid fibrils (curli), forming a cellulose matrix facilitated by FimH.

Using another mouse model and organism, John Gunn presented research on biofilms in the gallbladder (11). A percentage of people are colonized with *S. enterica* serovar Typhi in the gallbladder and go on to develop into asymptomatic, chronic carriers. People with gallstones have an increased risk for *S.* Typhi carriage. Transposon mutants of *S. enterica* serovar Typhimurium were screened for impaired adherence and biofilm formation on cholesterol-coated surfaces. Forty-nine mutants with a biofilm phenotype showed that genes involved in flagellum biosynthesis and structure primarily mediated attachment to cholesterol. Brian Peters presented fascinating work on mixed-species biofilms formed by the polymorphic fungus *C. albicans* and the bacterium *S. aureus* (31). Both can be coisolated from virtually all human mucosal sites and are responsible for diverse localized and deep-seated infections. Confocal microscopy and fluorescence *in situ* hybridization (FISH) showed not only that *S. aureus* possesses an affinity for the pathogenic hyphal form of *C. albicans in vitro* but that association with hyphae enhanced the invasion of epithelial cells. Proteomic analysis indicated that coculture of these organisms results in the upregulation of several virulence proteins in *S. aureus*, including Ldh1 and CodY, and suggests a novel mechanism of invasive *S. aureus* infection in the context of polymicrobial biofilm communities in the oral cavity.

BIOFILMS IN MEDICAL AND DENTAL INFECTIONS II

The second session on clinically relevant biofilms was chaired by Jeff Leid and Mark Shirtliff. The session began with Michael Givskov showing data on the ability of quorum sensing in *P. aeruginosa* to activate the production of rhamnolipids that concentrate in the matrix of the biofilm. These rhamnolipids result in a PMN leukocyte shield that is able to lyse incoming immune cells of the host, thereby preventing their function (1). While other investigators have shown that rhamnolipid production is reduced by AlgR in *in vitro* biofilms, he showed that inhibition is relieved *in vivo* and *in vitro* in response to PMNs. As a result, the penetrating neutrophils lyse, leaving behind host cellular debris and DNA, further protecting *P. aeruginosa* from clearance. A clinician, Sandeep Kathju, presented evidence of chronic biofilm infection on multiple forms of indwelling medical materials in his presentation (23). This ability to cause infection that can only be resolved by removal of the infected materials and not through antimicrobial therapy alone once again confirms decades of clinical experience with regard to biofilm infection.

The oral cavity demonstrates significant interspecies cooperation between microbes in a biofilm mode of growth (Fig. 1). This cooperation can be to such a level that the cooperating species cannot be cultured alone in salivary growth medium. By identifying those species that directly coaggregate from *in vivo* samples using quantum dot-conjugated antibodies, Paul Kolenbrander's lab was able extract specific cells via micromanipulation and reconstitute these cells for *in vitro* growth. While this was done with dual species, Dr. Kolenbrander also showed two examples of trinary culture biofilms and the codependence of the species on one another for *in vitro* growth in salivary medium (35).

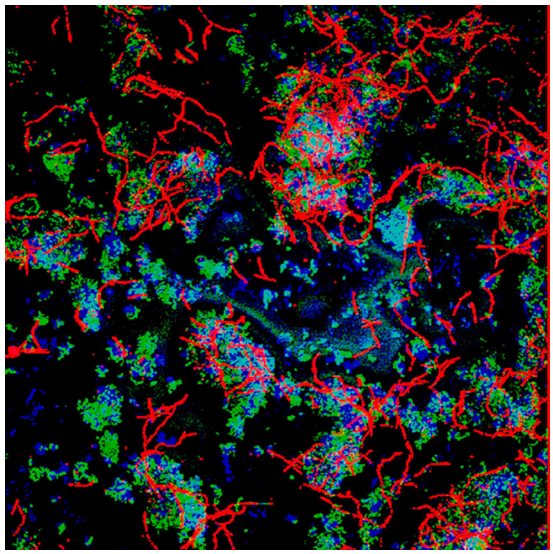

FIG. 1. Micrograph, taken at 18 h, of a multispecies biofilm grown in flow cells. Fn, *Fusobacterium nucleatum* (stained red); Aa, *Aggregatibacter actinomycetemcomitans* (stained green); Va, *Veillonella* sp. (stained dark blue); So, *Streptococcus oralis* (stained light blue). Courtesy of Paul Kolenbrander.

Annette Moter introduced an interesting new molecular tool available to researchers called Spacer-FISH and its use in surveying endocarditis biofilms (29). In regular FISH protocols, the measurement of viability is somewhat confounded because the probes hybridize to the 16S rRNA subunit of bacterial ribosomes. Measurement of viability and metabolic activity may be problematic using FISH alone since ribosomes can often stay intact even in recently nonviable and/or nonmetabolically active cells. Spacer-FISH directs probes against the intergenic spacer region (ISR) between the 16S and 23S rRNA segments prior to the cleavage and incorporation of these two subunits into ribosomes. Therefore, the ISR is short lived and detection of it via Spacer-FISH will give an indication of not only the viability but also the metabolic activity of the cell. The final speaker of the session, Jonathan Lellouche, showed that coating a compound with MgF_2 made it able to target the microbial membrane, disturbing membrane integrity, thereby demonstrating significant antimicrobial effects. Although MgF_2 is toxic to eukaryotic cells, it is apparent that an MgF_2 coating would have a number of useful industrial applications (25).

BIOFILM RESISTANCE MECHANISMS AND CONTROL STRATEGIES

Phil Bremer and John Thomas were the chairs of this session focused on the long-time observation that biofilms are notoriously difficult to treat with antimicrobials (16). Phil Stewart started his presentation proposing that in many instances cell/biofilm removal is as important as killing the resident cells and therefore there is a need to develop strategies to remove biofilms based on disrupting biofilm adhesion. Exposure of *Staphylococcus epidermidis* biofilms to 0.5 M urea or dispersin B for

15 min decreased biofilm stability and resulted in loss of material. In contrast, exposure to 0.1 M $FeCl_2$ or chlorhexidine resulted in a more stable biofilm than that in the untreated control. By combining treatments that weaken biofilm cohesion with physical forces such as fluid shear, it is possible to enhance biofilm removal.

Jeff Kaplan first outlined how the extracellular matrix that surrounds cells within a biofilm contains polymers such as DNA and polysaccharides. Enzymes that degrade matrix polymers and alter the physical integrity of the biofilm have been shown to inhibit biofilm formation and to either detach preformed biofilms or sensitize them to killing by antimicrobial agents, bacteriophages, or macrophages *in vitro*. The presentation focused on two well-studied matrix-degrading enzymes, the glycoside hydrolase dispersin B and DNase I, which cleaves DNA (22). In an *S. epidermidis* biofilm flow system, exposure to dispersin B resulted in an immediate decrease in the number of CFU recovered; however, after a few days, the number of CFU recovered returned to preexposure levels. Exposure of the biofilm to both dispersin B and rifampin eliminated the biofilm and sterilized the flow cell. Dr. Kaplan also discussed how DNase I sensitizes *S. aureus* biofilms to killing by a wide range of antimicrobial agents.

Hilary Lappin-Scott started off by posing the question of what is the goal of biofilm control—death or removal? She then commented that neither goal is readily achievable and postulated that a new way forward may be prevention of the undesirable aspects of biofilm development. Furthermore, Dr. Lappin-Scott pointed out that the role of nanoscience in microbiology needs to be assessed. Nanoparticles could be a new delivery mechanism for antimicrobial agents or vaccines that could disrupt biofilms; however, consideration needs to be given to the behavior of nanoparticles in ecosystems and their long-term effects (14).

C-DI-GMP SIGNALING

Susanne Häussler and Dan Wozniak convened this session. This session focused on the role of a ubiquitous intracellular messenger in eubacteria known as c-di-GMP. Levels of c-di-GMP are modulated in the cell by the opposing actions of diguanylate synthases (DGC) that synthesize c-di-GMP and phosphodiesterases (PDE), which degrade c-di-GMP. In bacteria, high intracellular levels of c-di-GMP promote a sessile lifestyle, which includes the expression of adhesion factors and EPSs. Conversely, low levels of c-di-GMP result in single-cell activities such as motility and dispersion. Presenters in this session focused on roles for DGC, PDE, and c-di-GMP in signaling and biofilm development in several diverse Gram-negative bacteria.

Fitnat Yildiz informed us that biofilm formation in *Vibrio cholerae* is positively regulated by VpsR and VpsT and negatively regulated by HapR and Crp. Transcriptome analysis identified DGC and PDE genes whose transcription is controlled by these core biofilm regulators (5). Complicating matters, the *V. cholerae* genome has 62 genes encoding putative proteins that can make or degrade c-di-GMP. Mutants with in-frame deletions of the genes were generated and analyzed for c-di GMP phenotypes. This analysis identified key DGCs, as well as a putative c-di-GMP binding protein. Susanne Häussler presented work on the use of a c-di-GMP-coupled Sepharose which was applied in pull-down experiments in order to identify novel c-di-GMP biding proteins. To date, only a few types of c-di-GMP effector molecules are known (e.g., PilZ domain proteins, degenerate PDE domains, and RXXD motif proteins). The use of immobilized c-di-GMP is expected to significantly contribute to the identification of novel c-di-GMP binding proteins that act as important effector proteins involved in the switch from the motile, virulent form to the sessile form of bacterial life within biofilms. Carrie Harwood described a system of c-di-GMP control of biofilm formation in *P. aeruginosa*. Dr. Harwood provided information that c-di-GMP levels modulate the expression of the biofilm-associated EPS Pel at two levels. First, c-di-GMP binding to the transcription factor FleQ reduces FleQ binding to the *pel* promoter, resulting in derepression of *pel* transcription (20). In addition, c-di-GMP modulates the activity of a Pel biosynthetic enzyme, PelD. It was shown that the Wsp signal transduction system (specifically, WspR) senses a signal associated with growth on a surface and transmits this information to adjust cellular levels of c-di-GMP, which is required for both *pel* transcription and Pel polysaccharide biosynthesis. A model was presented suggesting that activated WspR localized near the Pel biosynthetic machinery (PelD) or near FleQ. Diane McDougald discussed a mechanism of nitric oxide (NO)-mediated biofilm dispersal in *P. aeruginosa*. Prior work showed that NO increased *P. aeruginosa* motility, suggesting a link with c-di-GMP signaling (3). NO treatment also increased the sensitivity of biofilms to treatment with a range of antimicrobials. This was investigated by performing direct quantification of intracellular c-di-GMP levels, enzymatic assays to measure c-di-GMP PDE activity, and PDE inhibitor studies. Indeed, addition of NO stimulated PDE activity, decreased pools of c-di-GMP, and enhanced the dispersion of *P. aeruginosa* biofilms. Genetic and transcriptional profiling studies identified a chemotaxis-like regulator, BdlA, which indirectly regulates c-di-GMP levels and is required for NO-dependent biofilm dispersion.

Jason Tuckerman discussed the DosC-DosP system, which is involved in a pathway of c-di-GMP-dependent control of RNA degradosomes in *E. coli*. DosC is a DGC, while DosP is a PDE, and both proteins contain heme, which functions as the oxygen sensor (44). Tuckerman provided biochemical evidence that DosC and DosP copurify as part of a high-molecular-weight complex that includes components of the RNA degradosome core, providing a physiological link between c-di-GMP levels and oxygen-dependent control of RNA processing.

ENVIRONMENTAL BIOFILMS

The works presented in the session on environmental biofilms emphasized the interplay between bacterial communities and the characteristics of the environment surrounding those communities. Some of the works highlighted how the characteristics of the environment near sessile bacteria could affect their morphology, composition, and activity, and other works demonstrated how biofilm communities can interfere with the surrounding's features. The session was chaired by Maria Pereira and Diane McDougald.

In the first talk, Lars Dietrich showed that redox-active phenazines impact the structural organization of biofilms (12).

FIG. 2. A corrugated 7-day-old colony of *P. aeruginosa* defective in the production of phenazines. This strain exhibited hyperbiofilm production due to increased production of the polysaccharide Pel. The bacteria are stained with the dye Congo red. Courtesy of Lars Dietrich.

It was found that *P. aeruginosa* and *Streptomyces coelicolor* phenazine mutants give rise to sessile cells bigger and more wrinkled than the ones collected from wild-type biofilms (Fig. 2). Based on these results, it was concluded that phenazines are not only virulence factors inhibiting competing microorganisms but also play an important role in sessile bacterial communities. It was postulated that wrinkled colonies seemed to be an adaptive response of bacteria to overcome electron acceptor limitation. The effects of temperature, nutrients, and light in the evolutionary history of the species and the speciation of hot spring cyanobacterial mat communities were discussed by David Ward (48). Gradients of genetic variation were found in each major mat phylogenetic group occurring along environmental gradients. The use of internal transcribed spacer regions for analysis allowed exploration of the genetic variation within defined 16S rRNA genotypes. For example, it was shown that each 16S rRNA lineage contains about 12 ecological species.

In her talk, Sara Vetter discussed two models of flea-born transmission of *Yersinia pestis* plague related to biofilms: the classical blockage model and the relatively new early-phase transmission (EPT) model (13). The EPT model was described as an additional model to explain rapid transmission in unblocked fleas. Based on the results of analysis of the *hms* locus in *Y. pestis*, it was concluded that biofilm formation is not required for the early phase of *Y. pestis* transmission from fleas to hosts, but it is needed to maintain infection, being thus important for long-term infection of fleas.

Otto Ortega-Morales found extensive biofilms composed of phototrophic cyanobacteria, microalgae, bacteria, and fungi on exterior surfaces of buildings at the Kukulkan site of the Mayan archaeological site Chichen Itza. Biofilm formation on Mayan monuments is worrying since these sessile polymicrobial communities can exert deteriogenic activity (38). Elanna Bester discussed the effect of carbon limitation on biofilm activity and structure and the planktonic cell yield. She presented evidence that under environmental conditions that favor metabolically active *Pseudomonas* sp. biofilms, the planktonic cell yield from the biofilm is significantly enhanced, whereas the yield is maintained at diminished levels when environmental conditions are less favorable.

EMERGING TECHNOLOGIES FOR STUDYING STRUCTURED SYSTEMS

A session on new technologies for studying structured systems was chaired by Sarah Codd and Howard Ceri. Holger Daims described the use of single-cell methods that are applicable for multiple species. Two novel techniques are Raman-FISH, a combination of Raman microspectroscopy and FISH, and secondary ion mass spectrometry, which has very high sensitivity and spatial resolution (46, 49). Both methods detect the incorporation of isotope-labeled substrates by biofilm microbes without the need to cultivate these organisms.

Several specific applications were presented, including the degradation of naphthalene in contaminated groundwater and *in situ* analysis of the metabolism of environmental chlamydiae. Mike Franklin presented a microdissection adaptation to "-omics" techniques. Traditionally, "-omic" measurements are averaged over the whole biofilm and this masks the spatial heterogeneity inherent to biofilms. Franklin's group has developed a way to perform transcriptional analysis of biofilm subpopulations that have been selectively harvested from a small region of a biofilm (27). The method involves cryoembedding of the biofilm sample, isolating a region by laser capture microdissection, and then using quantitative reverse transcription-PCR to study the expression of specific genes (26). Franklin presented results where samples from a stratified biofilm showed high expression of the housekeeping gene *acpP* at the top of biofilms and no expression in the middle and bottom layers. Similar analyses of the *rpoS*, *rhlR*, and 16S rRNA genes were performed.

Alfred Spormann looked at energy dependence as an important component of biofilm stability and if *de novo* transcription and translation are requisite for cell detachment. To address the importance of energy in maintaining biofilm integrity, stopped-flow assays that reduced oxygen levels or metabolic inhibition were combined with reporters dependent upon energy levels to show the association of energy levels with cell loss from the biofilm of *Shewanella oneidensis* (42). Reduced ATP levels and decreased c-di-GMP levels were therefore associated with massive cell loss. Similar results were seen with the *mshA* and *mxdB* deletions decoupling the observation from type IV pilus or carbohydrate production. Biofilm age was, however, seen to impact this phenotype.

Nikolai Stankiewicz reported on a new technology for *in vivo* RNA labeling and separation (39). In this technique, digoxigenin-11-UTP is effectively incorporated into *de novo* RNA synthesis by both Gram-positive and -negative cell populations. Labeled RNA can be separated by the use of biotinylated antidigoxigenin antibody and streptavidin-functionalized magnetic beads, which resulted in high levels of both purification and specificity. This procedure makes it possible to measure specific mRNA synthesis *in situ*, which would be useful in a number of experimental scenarios.

GENETIC DIVERSITY AND DIVISION OF LABOR

Roberto Kolter gave the last keynote address, introducing the audience to the last session: diversification and division of labor in biofilms. His worked focused on colonies of *B. subtilis* grown on solid medium. He identified several subpopulations within colonies, such as miners, matrix builders, and cannibals (28). These cell types exhibit extensive extracellular communication over the course of colony growth through the sensing of self-generated signals. The signals activate a set of sensor kinases which, in turn, phosphorylate three major regulators, Spo0A, DegU, and ComA. Each phosphorylated regulator activates a specific differentiation program while at the same time repressing other differentiation programs, allowing cells to differentiate in response to a specific cue, even in the presence of other signals. Differentiation of distinct cells types in *B. subtilis* is necessary for the proper development of the bacterial community.

The last session of the conference was chaired by Dao Nguyen and Garth Ehrlich. Ehrlich presented evidence on the remarkable levels of genomic plasticity and degree of horizontal gene transfer in *Streptococcus pneumoniae*, an important human pathogen (17). Through comparative genomic analysis of 17 sequenced *S. pneumoniae* strains, a "supragenome" was reconstructed. This universal set of genes is larger than the genome of any single bacterium and highlights the enormous genetic resources that can be used by the natural competent species *S. pneumoniae* through genetic recombination. Tim Tolker-Nielsen showed that the formation of mushroom-like biofilm structures requires the sequential involvement and interaction of various bacterial subpopulations. In this system, distinct *P. aeruginosa* subpopulations produce factors, e.g., pyoverdine and PQS, required for the development of another subpopulation that does not produce these factors. Since flat biofilms lacking mushroom structures are also less tolerant of biocides and antibiotics, knowledge of the molecular mechanisms underlying the evolution of physiologically distinct subpopulations in biofilms might be the basis for the development of novel antibiofilm treatment strategies.

As part of its life cycle, *V. cholerae* lives in the aquatic environment, attached to chitin-containing exoskeleton surfaces of crustaceans. Melanie Blokesch found that chitin induces the expression of 41 genes involved in chitin colonization and utilization. Most surprisingly, chitin also induces natural competence, a process previously never shown in *V. cholerae*. This natural competence program requires a Tfox-mediated signal transduction cascade, as well as type IV pilus-mediated uptake of DNA. It is also dependent on quorum sensing, as well as the cyclic AMP receptor protein, a signal induced under nutrient limitation or stress. The ability of *V. cholerae* to be competent for natural transformation allows it to take up free DNA from its environment when attached to chitin surfaces (7). This may have broad-ranging consequences for the genetic diversification of *V. cholerae*, and chitin-attached *V. cholerae* communities may represent an evolutionary hot spot for this pathogen.

Gerard Wong has developed a novel automated biometric analysis tool to study time-lapse confocal microscopy movies of single cells. State-of the art image recognition and particle tracking algorithms are used to track hundreds of surface-associated cells (~800) at a time. The cell's orientation, projected length, and velocity are inferred from an automated image analysis. As an example, his group studied type IV pilus-mediated motility in *P. aeruginosa*. Distinct patterns of pilus-mediated motility such as "crawling" and "walking" were characterized. The flagellum, on the other hand, was involved in swimming and spinning motility. This novel integration of image analysis allows the study of surface-associated motility at a resolution and level of precision previously not possible.

RSCV of *P. aeruginosa* are frequently isolated from cystic fibrosis infections, as well as *in vitro* biofilms (41). This phenotype has been associated with increased second-messenger c-di-GMP, including through the well-characterized *wsp* pathway. In order to identify novel pathways involved in the RSCV phenotype, Joe Harrison performed a transposon mutagenesis screen in the delta *wspR* background. After screening over 75,000 clones, 24 different genes were identified belonging to three distinct pathways: orphan diguanylate cyclases and PDE, flagellum biosynthesis, and the regulator of secondary metabolism pathway. This suggests that multiple genetic pathways can lead to the RSCV phenotype.

CONCLUSION

There was a consensus of the attendees of the Biofilm 2009 meeting in Cancun that the high scientific quality and interdisciplinary nature of the biofilm research presented deservedly attracted the international scientific community and significantly stimulated the discussions. Exciting science in a field with broad horizons will continue to pave the way toward a novel understanding of bacterial community behavior and will probably also put new emphasis on alternative therapeutic strategies in human diseases. The biofilm organization committee has assembled an attractive, scientifically relevant, and diverse program and hopes that the biofilm conference series will continue to attract young, as well as advanced, investigators and to provide the basis for an effective interchange of scientific ideas.

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REFERENCES

1. **Alhede, M., T. Bjarnsholt, P. O. Jensen, R. K. Phipps, C. Moser, L. Christophersen, L. D. Christensen, M. van Gennip, M. Parsek, N. Hoiby, T. B. Rasmussen, and M. Givskov.** 2009. *Pseudomonas aeruginosa* recognizes and responds aggressively to the presence of polymorphonuclear leukocytes. Microbiology **155:**3500–3508.
2. **Bakaletz, L. O.** 2007. Bacterial biofilms in otitis media: evidence and relevance. Pediatr. Infect. Dis. J. **26**(10 Suppl.)**:**S17–S19.
3. **Barraud, N., D. Schleheck, J. Klebensberger, J. S. Webb, D. J. Hassett, S. A. Rice, and S. Kjelleberg.** 2009. Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. J. Bacteriol. **191:**7333–7342.
4. **Bester, E., O. Kroukamp, G. M. Wolfaardt, L. Boonzaaier, and S. N. Liss.** 2010. Metabolic differentiation in biofilms as indicated by carbon dioxide production rates. Appl. Environ. Microbiol. **76:**1189–1197.
5. Reference deleted.
6. **Blair, K. M., L. Turner, J. T. Winkelman, H. C. Berg, and D. B. Kearns.** 2008. A molecular clutch disables flagella in the Bacillus subtilis biofilm. Science **320:**1636–1638.
7. **Blokesch, M., and G. K. Schoolnik.** 2008. The extracellular nuclease Dns and its role in natural transformation of *Vibrio cholerae*. J. Bacteriol. **190:**7232–7240.

8. **Bradley, R. B., D. G. Aaron, M. Keiji, S. Ram, J. W. Daniel, and R. P. Matthew.** 2010. *Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. Mol. Microbiol. **75:**827–842.
9. **Cash, H. L., C. V. Whitham, C. L. Behrendt, and L. V. Hooper.** 2006. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science **313:**1126–1130.
10. **Conibear, T. C. R., S. L. Collins, and J. S. Webb.** 2009. Role of mutation in *Pseudomonas aeruginosa* biofilm development. PLoS One **4:**e6289.
11. **Crawford, R. W., R. Rosales-Reyes, M. de la Luz Ramírez-Aguilar, O. Chapa-Azuela, C. Alpuche-Aranda, and J. S. Gunn.** 2010. Gallstones play a significant role in *Salmonella* spp. gallbladder colonization and carriage. Proc. Natl. Acad. Sci. U. S. A. **107:**4353–4358.
12. **Davies, D. G., and C. N. H. Marques.** 2009. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J. Bacteriol. **191:** 1393–1403.
13. **Dietrich, L. E. P., T. K. Teal, A. Price-Whelan, and D. K. Newman.** 2008. Redox-active antibiotics control gene expression and community behavior in divergent bacteria. Science **321:**1203–1206.
14. **Eisen, R. J., J. N. Borchert, J. L. Holmes, G. Amatre, K. Van Wyk, R. E. Enscore, N. Babi, L. A. Atiku, A. P. Wilder, S. M. Vetter, S. W. Bearden, J. A. Montenieri, and K. L. Gage.** 2008. Early-phase transmission of *Yersinia pestis* by cat fleas (*Ctenocephalides felis*) and their potential role as vectors in a plague-endemic region of Uganda. Am. J. Trop. Med. Hyg. **78:**949–956.
15. **Elswaifi, S. F., J. R. Palmieri, K. S. Hockey, and B. A. Rzigalinski.** 2009. Antioxidant nanoparticles for control of infectious disease. Infect. Disord. Drug Targets **9:**445–452.
16. **Franks, A. E., K. P. Nevin, R. H. Glaven, and D. R. Lovley.** 2010. Microtoming coupled to microarray analysis to evaluate the spatial metabolic status of *Geobacter sulfurreducens* biofilms. ISME J. **4:**509–519.
17. **Fux, C. A., J. W. Costerton, P. S. Stewart, and P. Stoodley.** 2005. Survival strategies of infectious biofilms. Trends Microbiol. **13:**34–40.
18. **Hickman, J. W., and C. S. Harwood.** 2008. Identification of FleQ from *Pseudomonas aeruginosa* as a c-di-GMP-responsive transcription factor. Mol. Microbiol. **69:**376–389.
19. **Hiller, N. L., B. Janto, J. S. Hogg, R. Boissy, S. Yu, E. Powell, R. Keefe, N. E. Ehrlich, K. Shen, J. Hayes, K. Barbadora, W. Klimke, D. Dernovoy, T. Tatusova, J. Parkhill, S. D. Bentley, J. C. Post, G. D. Ehrlich, and F. Z. Hu.** 2007. Comparative genomic analyses of seventeen *Streptococcus pneumoniae* strains: insights into the pneumococcal supragenome. J. Bacteriol. **189:**8186–8195.
20. **Hooper, L. V., and A. J. Macpherson.** 2010. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat. Rev. Immunol. **10:**159–169.
21. **Huang, W. E., K. Stoecker, R. Griffiths, L. Newbold, H. Daims, A. S. Whiteley, and M. Wagner.** 2007. Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence *in situ* hybridization for the single cell analysis of identity and function. Environ. Microbiol. **9:**1878–1889.
22. **Johnson, C. M., D. A. Manias, H. A. H. Haemig, S. Shokeen, K. E. Weaver, T. M. Henkin, and G. M. Dunny.** 2010. Direct evidence for control of the pheromone-inducible *prgQ* operon of *Enterococcus faecalis* plasmid pCF10 by a countertranscript-driven attenuation mechanism. J. Bacteriol. **192:**1634–1642.
23. **Kaplan, J. B.** 2010. Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. J. Dent. Res. **89:**205–218.
24. **Kathju, S., L. Nistico, L. Hall-Stoodley, J. C. Post, G. D. Ehrlich, and P. Stoodley.** 2009. Chronic surgical site infection due to suture-associated polymicrobial biofilm. Surg. Infect. (Larchmt.) **10:**457–461.
25. **Lauderdale, K. J., B. R. Boles, A. L. Cheung, and A. R. Horswill.** 2009. Interconnections between sigma B, *agr*, and proteolytic activity in *Staphylococcus aureus* biofilm maturation. Infect. Immun. **77:**1623–1635.
26. **Lellouche, J., E. Kahana, S. Elias, A. Gedanken, and E. Banin.** 2009. Antibiofilm activity of nanosized magnesium fluoride. Biomaterials **30:**5969–5978.
27. **Lenz, A. P., K. S. Williamson, B. Pitts, P. S. Stewart, and M. J. Franklin.** 2008. Localized gene expression in *Pseudomonas aeruginosa* biofilms. Appl. Environ. Microbiol. **74:**4463–4471.
28. **López, D., and R. Kolter.** 2010. Extracellular signals that define distinct and coexisting cell fates in *Bacillus subtilis*. FEMS Microbiol. Rev. **34:**134–149.
29. **Ma, L., M. Conover, H. Lu, M. R. Parsek, K. Bayles, and D. J. Wozniak.** 2009. Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. PLoS Pathog. **5:**e1000354.
30. **Mallmann, C., S. Siemoneit, D. Schmiedel, A. Petrich, D. M. Gescher, E. Halle, M. Musci, R. Hetzer, U. B. Göbel, and A. Moter.** 20 August 2009. Fluorescence *in situ* hybridization to improve the diagnosis of endocarditis: a pilot study. Clin. Microbiol. Infect. [Epub ahead of print.] doi:10.1111/j.1469-0691.2009.02936.x.
31. **Mann, E. E., K. C. Rice, B. R. Boles, J. L. Endres, D. Ranjit, L. Chandramohan, L. H. Tsang, M. S. Smeltzer, A. R. Horswill, and K. W. Bayles.** 2009. Modulation of eDNA release and degradation affects *Staphylococcus aureus* biofilm maturation. PLoS One **4:**e5822.
32. **Moreira, C. G., D. Weinshenker, and V. Sperandio.** 2010. QseC mediates *Salmonella enterica* serovar Typhimurium virulence in vitro and in vivo. Infect. Immun. **78:**914–926.
33. **Mulcahy, H., L. Charron-Mazenod, and S. Lewenza.** 2008. Extracellular DNA chelates cations and induces antibiotic resistance in *Pseudomonas aeruginosa* biofilms. PLoS Pathog. **4:**e1000213.
34. **Nemati, M., K. Hermans, L. A. Devriese, D. Maes, and F. Haesebrouck.** 2009. Screening of genes encoding adhesion factors and biofilm formation in *Staphylococcus aureus* isolates from poultry. Avian Pathol. **38:**513–517.
35. **Newell, P. D., R. D. Monds, and G. A. O'Toole.** 2009. LapD is a bis-(3′,5′)-cyclic dimeric GMP-binding protein that regulates surface attachment by *Pseudomonas fluorescens*. Proc. Natl. Acad. Sci. U. S. A. **106:**3461–3466.
36. **Paster, B. J., and F. E. Dewhirst.** 2009. Molecular microbial diagnosis. Periodontol. 2000 **51:**38–44.
37. **Periasamy, S., and P. E. Kolenbrander.** 2010. Central role of early colonizer *Veillonella* sp. in establishing multispecies biofilm communities with initial, middle, and late colonizers of enamel. J. Bacteriol. **192:**2965–2972.
38. **Petrova, O. E., and K. Sauer.** 2009. A novel signaling network essential for regulating *Pseudomonas aeruginosa* biofilm development. PLoS Pathog. **5:**e1000668.
39. **Scheerer, S., O. Ortega-Morales, and C. Gaylarde.** 2009. Microbial deterioration of stone monuments—an updated overview. Adv. Appl. Microbiol. **66:**97–139.
40. **Shirtliff, M. E., B. M. Peters, and M. A. Jabra-Rizk.** 2009. Cross-kingdom interactions: *Candida albicans* and bacteria. FEMS Microbiol. Lett. **299:**1–8.
41. **Stankiewicz, N., A. Gold, Y. Yüksel, S. Berensmeier, and T. Schwartz.** 2009. In vivo labeling and specific magnetic bead separation of RNA for biofilm characterization and stress-induced gene expression analysis in bacteria. J. Microbiol. Methods **79:**344–352.
42. **Starkey, M., J. H. Hickman, L. Ma, N. Zhang, S. De Long, A. Hinz, S. Palacios, C. Manoil, M. J. Kirisits, T. D. Starner, D. J. Wozniak, C. S. Harwood, and M. R. Parsek.** 2009. *Pseudomonas aeruginosa* rugose small-colony variants have adaptations that likely promote persistence in the cystic fibrosis lung. J. Bacteriol. **191:**3492–3503.
43. **Thormann, K. M., S. Duttler, R. M. Saville, M. Hyodo, S. Shukla, Y. Hayakawa, and A. M. Spormann.** 2006. Control of formation and cellular detachment from *Shewanella oneidensis* MR-1 biofilms by cyclic di-GMP. J. Bacteriol. **188:**2681–2691.
44. **Toh, E., H. D. Kurtz, Jr., and Y. V. Brun.** 2008. Characterization of the *Caulobacter crescentus* holdfast polysaccharide biosynthesis pathway reveals significant redundancy in the initiating glycosyltransferase and polymerase steps. J. Bacteriol. **190:**7219–7231.
45. **Tuckerman, J. R., G. Gonzalez, E. H. S. Sousa, X. Wan, J. A. Saito, M. Alam, and M.-A. Gilles-Gonzalez.** 2009. An oxygen-sensing diguanylate cyclase and phosphodiesterase couple for c-di-GMP control. Biochemistry **48:**9764–9774.
46. **Van Houdt, R., and C. W. Michiels.** 2005. Role of bacterial cell surface structures in *Escherichia coli* biofilm formation. Res. Microbiol. **156:**626–633.
47. **Wagner, M.** 2009. Single-cell ecophysiology of microbes as revealed by Raman microspectroscopy or secondary ion mass spectrometry imaging. Annu. Rev. Microbiol. **63:**411–429.
48. **Waksman, G., and S. J. Hultgren.** 2009. Structural biology of the chaperone-usher pathway of pilus biogenesis. Nat. Rev. Microbiol. **7:**765–774.
49. **Ward, D. M., F. M. Cohan, D. Bhaya, J. F. Heidelberg, M. Kuhl, and A. Grossman.** 2008. Genomics, environmental genomics and the issue of microbial species. Heredity **100:**207–219.
50. **Wolcott, R. D., and G. D. Ehrlich.** 2008. Biofilms and chronic infections. JAMA **299:**2682–2684.
51. **Xiao, J., and H. Koo.** 4 November 2009. Structural organization and dynamics of exopolysaccharide matrix and microcolonies formation by *Streptococcus mutans* in biofilms. J. Appl. Microbiol. [Epub ahead of print.] doi: 10.1111/j.1365-2672.2009.04616.x.
52. **Yang, L., M. Nilsson, M. Gjermansen, M. Givskov, and T. Tolker-Nielsen.** 2009. Pyoverdine and PQS mediated subpopulation interactions involved in *Pseudomonas aeruginosa* biofilm formation. Mol. Microbiol. **74:**1380–1392.
53. **Zhou, X., C. J. Brown, Z. Abdo, C. C. Davis, M. A. Hansmann, P. Joyce, J. A. Foster, and L. J. Forney.** 2007. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. ISME J. **1:**121–133.